This report is required by law (7 USC 2143). Failure to report according to the regulations can result in an order to cease and desist and to be subject to penalties as provided for in Section 2150.

See reverse side for additional information.

Interagency Report Control No 0180-DOA-AN

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

ANNUAL REPORT OF RESEARCH FACILITY
(TYPE OR PRINT)

1. REGISTRATION NO. 55-R-0001
CUSTOMER NO. 825

FORM APPROVED
OMB NO. 0579-0036

2. HEADQUARTERS RESEARCH FACILITY *(Name and Address, as registered with USDA, include Zip Code)*

WAKE FOREST UNIVERSITY
MEDICAL CENTER BOULEVARD
WINSTON SALEM, NC 27109

DEC 0 1 2006

(b)(6), (b)(7)c

3. REPORTING FACILITY (List all locations where animals were housed or used in actual research, testing, teaching, or experimentation, or held for these purposes. Attach additional sheets if necessary.)

FACILITY LOCATIONS*(sites)*

See Attached Listing

(b)(2)High, (b)(7)f

(b)(2)High, (b)(7)f

REPORT OF ANIMALS USED BY OR UNDER CONTROL OF RESEARCH FACILITY *(Attach additional sheets if necessary or use APHIS FORM 7023A.)*

A. Animals Covered By The Animal Welfare Regulations	B. Number of animals being bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery but not yet used for such purposes.	C. Number of animals upon which teaching, research, experiments, or tests were conducted involving no pain, distress, or use of pain-relieving drugs.	D. Number of animals upon which experiments, teaching, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs were used.	E. Number of animals upon which teaching, experiments, research, surgery or tests were conducted involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic, analgesic, or tranquilizing drugs would have adversely affected the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests. *(An explanation of the procedures producing pain or distress in these animals and the reasons such drugs were not used must be attached to this report)*	F. TOTAL NO. OF ANIMALS (Cols. C + D + E)
4. Dogs			80		80
5. Cats			104		104
6. Guinea Pigs			0		0
7. Hamsters			0		0
8. Rabbits			176		176
9. Non-Human Primates		104	792	18	914
10. Sheep			229		229
11. Pigs			34		34
12. Other Farm Animals					
13. Other Animals Bats		17			17
Squirrels		60			60
Ferrets		124	58		182
Chinchillas			39		39

ASSURANCE STATEMENTS

1) Professionally acceptable standards governing the care, treatment, and use of animals, including appropriate use of anesthetic, analgesic, and tranquilizing drugs, prior to, during, and following actual research, teaching, testing, surgery, or experimentation were followed by this research facility.

2) Each principal investigator has considered alternatives to painful procedures.

3) This facility is adhering to the standards and regulations under the Act, and it has required that exceptions to the standards and regulations be specified and explained by the principal investigator and approved by the Institutional Animal Care and Use Committee (IACUC). **A summary of all the exceptions is attached to this annual report.** In addition to identifying the IACUC-approved exceptions, this summary includes a brief explanation of the exceptions, as well as the species and number of animals affected.

4) The attending veterinarian for this research facility has appropriate authority to ensure the provision of adequate veterinary care and to oversee the adequacy of other aspects of animal care and use.

[redacted]

CERTIFICATION BY HEADQUARTERS RESEARCH FACILITY OFFICIAL
(Chief Executive Officer or Legally Responsible Institutional official)

I certify that the above is true, correct, and complete (7 U.S.C. Section 2143)

INSTITUTIONAL OFFICIAL

NAME & TITLE OF C.E.O. OR INSTITUTION

(b)(6), (b)(7)c

[redacted]

DATE SIGNED 11-28-06

ADQUARTERS

Attachment to APHIS FORM 7023

Summary of Exceptions to Regulations and Standards
Wake Forest University 55-R-0001
Fiscal Year 2005-2006

1. The following is an exception to the requirements for sanitizing nonhuman primate primary enclosures at required frequencies:

(b)(2)High, (b)(7)f is used to house nonhuman primates imported directly from foreign sources (Indonesia). As required by the Centers for Disease Control (CDC), cages used in this building can not be sent through the mechanical cage washer until after the first thirty-one days (Phase I) of quarantine. Animal waste is removed from cage pans daily. After monkeys have completed Phase I quarantine, cages are sanitized at intervals in accordance with federal regulations and standards.

2. The following protocols involve an exception to the AWA regulation limiting animals to one protocol with a major survival operative procedure.

a. *A 05-047: Estrogen/Phytoestrogen Interactions: Uterine Effects*
The animals used in this study are from a protocol in which they received a major surgery (ovariectomy) four years ago. Because a second surgery was not described in the initial protocol and is considered a major revision by our committee, a new protocol was required. The sole purpose of the new protocol is to seek permission to perform a hysterectomy on these animals, while pursuing the same scientific aims as the original protocol. The previous work has shown that isoflavones in dietary soybeans can protect against the cancer-causing effect of estrogen in the breast and possibly the uterus. However there is a possibility that the compounds themselves cause precancerous changes. Indeed, recent studies of women have increased the urgency of evaluating the uterus. The uteri of the animals in this study have been evaluated for 4 years. However, the original method of uterine evaluation (ultrasound) is not sensitive enough to detect small changes in the endometrium. This proposal involves removing the uterus so that it can be examined microscopically. Tissue sampling cannot be done in any other way because of the anatomy of the entrance to the uterus in this species. A written request was made to and approved by the Deputy Administrator, Animal Care, APHIS, USDA, on May 9, 2005.

Species Used: Female cynolmolgus monkeys (*Macaca fascicularis*)
Number Used: 30

b. *A 05-178: Diencephalic mechanisms of visuomotor integration: neuroanatomical studies*
The intent is study the integration of visual sensory and motor information in the brain for the eventual treatment of visual dysfunction in conditions such as head trauma, stroke and Parkinson's disease. As part of a previous protocol, the investigators have recorded activity in single neurons within oculomotor thalamus that characterizes the integration of sensory (visual) and motor (eye movement) information necessary for behavior. A more complete understanding requires the correlation of recording sites with anatomical data from the same animals. Therefore, an additional major surgical procedure is proposed to allow the placement of neuroanatomic tracers.

At present, only a single anatomical tracer is available with the required characteristics: Phaseolus vulgaris leucoagglutinin, which is delivered iontophoretically through fine fragile glass micropipettes that cannot be lowered through 25 mm of cortical tissue to subcortical targets. Instead, a major survival surgical procedure must be performed for focal cortical removal to visualize subcortical targets. Moreover, a 14 day post-injection survival period is required. *This approach ultimately results in the use of fewer animals than the alternative of starting from the beginning with entirely new animals to conduct the joint electrophysiology and neuroanatomic studies.* A written request was made to and approved by the Deputy Administrator, Animal Care, APHIS, USDA, on December 1, 2005.

Species Used: Monkeys (Macaca Mulatta)
Number Used: 4

3. All of the following protocols involve exceptions to the cage size requirements for sheep primary enclosures. Each exception was approved by the Wake Forest University (WFU) Instituitional Animal Care and Use Committee (IACUC), after their determination of scientific justification. For each protocol listed, sheep *(Ovis aries)* are used in studies of pituitary-adrenal hormones, renin, and vasopressin, and are housed in metabolism carts for up to fourteen days. These studies are important to the understanding of factors relating to the health and survival of newborn infants. The Committee's justification for the exception is based on data that show movement of animals in and out of the carts on a daily basis would adversely influence the hormones measured in these experiments.

A 02-196/A 06-003: Prenatal and Neonatal Pituitary-Adrenal Function, Number Used: 22
A 03-148: Prenatal Glucocorticoid and Postnatal Blood Pressure, Number Used: 32
A 04-134: Fetal Programming and Salt Sensitivity, Animal Care, Number Used: 4
A 05-078: Project 3: Antenatal steroid exposure and neurogenic control of blood pressure, Number Used: 4
A 05-105: Vulnerability of the Fetal Brain to Hypoxia-Ischemi, Number Used: 5

4. The following protocols involve exceptions to the requirements for watering nonhuman primates. For each protocol listed, full access to water is regulated in a manner to ensure animals are adequately motivated to perform traditional operant conditioning paradigms. Each exception was approved by the WFU IACUC, after their determination of scientific justification.

a. *A 03-075/A 06-122: Diencephalic Mechanisms of Visuomotor Integration*
Access to water is regulated such that the first fluid intake of the day will occur during a training or experimental session. A well-trained animal will work to satiety during an experimental session and, in doing so, obtain its minimum daily water requirement. In the event that an animal does not obtain its minimum fluid intake during a session, fluid will be supplemented to the appropriate level when the animal is returned to its home cage. Typically, water regulation is begun on Sunday in preparation for behavioral testing on Monday. Animals participate in experiments on Monday through Friday and receive ad libitum access to water on Friday afternoon through Sunday morning. Throughout the duration of an individual animal's participation in experimental protocols, fluid intake, weight, and a variety of other physiological and behavioral measures are recorded to assure the animal's continued health and well-being. Animals are weighed daily (on the days they are tested). Health and hydration are assessed every day by both research and veterinary personnel. These studies are important to understanding the neural bases of sensory and motor control of the mechanisms of decision making in humans.

Species Used: Male rhesus monkeys (*Macaca mulatta*)
Number Used: 4

b. *A 04-050: Cortical Implications for Rehabilitation*
Access to water is regulated such that the first fluid intake of the day will occur during a training or experimental session. A well-trained animal will work to satiety during an experimental session and, in

doing so, obtain its minimum daily water requirement. In the event that an animal does not obtain its minimum fluid intake during a session, fluid will be supplemented to the appropriate level when the animal is returned to its home cage. Typically, water regulation is begun on Sunday in preparation for behavioral testing on Monday. Animals participate in experiments on Monday through Friday and receive ad libitum access to water on Friday afternoon through Sunday morning. Throughout the duration of an individual animal's participation in experimental protocols, fluid intake, weight, and a variety of other physiological and behavioral measures are recorded to assure the animal's continued health and well-being. Animals are weighed daily (on the days they are tested). Health and hydration are assessed every day by both research and veterinary personnel. Findings from this study will aid in improving rehabilitive therapy for individuals following a stroke.

Species Used: Male rhesus monkeys (*Macaca mulatta*)
Number Used: 4

c. *A 05-165: Cross-Modal Integration in Primate Midbrain*
Water regulation only occurs during periods of training and experimentation. Throughout the year animals are given ad lib access to water for variable, but often extended, periods of time (months). Prior to initiation of training, each animal's *ad lib* intake of fluid is measured each day to determine its normal daily fluid consumption; generally this is between 200-250 ml. Then, during training and experiments, the animal is allowed to work to satiety during a session. If the animal will not work for that long, which can occur particularly early in the training, its fluid intake is supplemented with either pieces of fruit (apple, orange) or Gatorade. On the weekends, each animal is given access to water *ad lib* in its cage. A daily record is made of the animal's fluid intake as well as the presence of urine and feces in the pan. Health and hydration are assessed every day by both research and veterinary personnel. These studies will provide information about the dynamics of brain function in multisensory integration from external stimuli, e.g., light and sound and eye movements.

Species Used: Male rhesus monkeys (*Macaca mulatta*)
Number Used: 4

d. *A 03-012/A 06-034: The Cortical Circuitry that Mediates Working Memory*
Water intake is regulated so that the animals' first fluid intake of the day occurs during a training or experimental session. Fully-trained animals work to satiety, obtaining more than their minimum fluid requirement. In the event that an animal does not obtain its minimum intake during a session (particularly during the early stages of training) it is supplemented after the end of the session by the investigator. Animals participate in experiments 5 days a week and have free access to water for two days per week. Prior to beginning training, each animal's ad lib intake of fluid is recorded to determine its normal daily consumption. This is generally in the range of 20-40 ml/kg. A daily record is made of the animal's fluid and food intake. Body weights are recorded at least twice per week (daily during experiments). Health and hydration are assessed every day by both research and veterinary personnel. Water regulation is temporarily suspended if a monkey exhibits signs of dehydration (i.e. decreased urine production, dry feces, anorexia, changes in skin elasticity). Findings from this project will offer insight into possible means of reversing the effects in humans of brain damage and mental illness.

Species Used: Male rhesus monkeys (*Macaca mulatta*)
Number Used: 3

e. *A 03-013/A 06-033: Distributed, Passive, and Active Components of Memory*

Water intake is regulated so that the animals' first fluid intake of the day occurs during a training or experimental session. Fully-trained animals work to satiety, obtaining more than their minimum fluid requirement. In the event that an animal does not obtain its minimum intake during a session (particularly during the early stages of training) it is supplemented after the end of the session by the investigator. Animals participate in experiments 5 days a week and have free access to water for two days per week. Prior to beginning training, each animal's ad lib intake of fluid is recorded to determine its normal daily consumption. This is generally in the range of 20-40 ml/kg. A daily record is made of the animal's fluid and food intake. Body weights are recorded at least twice per week (daily during experiments). Health and hydration are assessed every day by both research and veterinary personnel. Water regulation is temporarily suspended if a monkey exhibits signs of dehydration (i.e. decreased urine production, dry feces, anorexia, changes in skin elasticity). These studies will provide information about the biological basis of memory in healthy subjects, and suggest the means to modify and compensate for loss of memory function in stroke and brain trauma.

Species Used: Male rhesus monkeys (*Macaca mulatta*)
Number Used: 4

5. The following protocol involves an exception to the lighting and watering requirements for nonhuman primates. The exceptions were approved by the WFU ACUC, after determination of scientific justification.

A 05-136: Neurophysiological Recording from Nonhuman Primate Brain
Full access to water is regulated in a manner to ensure animals are adequately motivated to perform traditional operant conditioning paradigms. Access to water is regulated such that the first fluid intake of the day will occur during a training or experimental session. A well-trained animal will work to satiety during an experimental session and, in doing so, obtain its minimum daily water requirement (as determined by a formula based on age, size and weight of the animal). In the event that the animal does not obtain its minimum fluid intake during a session, fluid is supplemented to the appropriate level when the animal is returned to its home cage. Typically, water regulation is begun on Sunday afternoon in preparation for behavioral testing on Monday. Animals participate in experiments on Monday through Friday and may receive additional water (or high-water-content fruit and vegetable supplements) on Friday afternoon through Sunday afternoon. Throughout the duration of an individual animal's participation in experimental protocols, fluid intake, body weight, behavioral performance, and observations of animal health are recorded daily. Periodic veterinary assessment, and other measures such as urine output, hematocrit, etc. are also used to assure the animals' continued health and well-being. Animals are weighed on the days they are tested (or at least weekly). Health and hydration are assessed every day by both research and veterinary personnel.

One of the aims of this study is to identify those brain areas most affected by brief (36-54 hour) sleep deprivation in an animal model. A monkey performs its behavioral/recording session normally during the day before sleep deprivation. That evening, before the lights go off in the housing area, the monkey is moved to a separate room in which the lights stay on all night and is kept awake by use of nonaversive stimuli (videos, music, food treats, toys, random computer sounds, interactions with technicians). The behavioral and electrophysiological testing of the monkey is then repeated the following afternoon. Sleep deprivation studies are performed at minimum 10 day intervals, typically only once per 2-4 weeks per monkey.

Species Used: Male rhesus monkeys (*Macaca mulatta*)
Number Used: 18

6. The following protocols involve exceptions to the lighting and space requirements for cats. The exceptions were approved by the WFU ACUC, after determination of scientific justification.

These studies examine the role of visual experience on the development of multisensory processes in the midbrain. From birth, kittens are raised to adulthood in total darkness. Total darkness is required to eliminate all visual experience during development in a reversible manner. Procedures such as enucleation or eyelid-suturing are inadequate because they are not reversible or allow a significant amount of light to penetrate the lids. A portion of the population is returned to full-spectrum lighting at six to twelve months of age. When in darkness, animals are cared for in accordance with all other standards and regulations. Infrared goggles are used by animal care, veterinary, and research personnel who enter the room daily to perform routine husbandry, health evaluations, and research manipulations. Criteria for monitoring the health and well-being of animals were developed, reviewed, and approved by the IACUC. All dark-reared animals have remained healthy and gain weight at rates similar to age-matched controls.

Animals in the room spend six hours per day together in an experimental pen which provides adequate space, but less than the minimum square footage per cat required by the USDA for standard housing. The size of the pen is a critical element in restricting the sensory experience of these animals, since it serves to constrain the minimum and maximum angular disparities of visual and auditory experience. Enlarging the size of the experimental pen would increase the maximal disparity beyond that which is believed to have an effect on reorganization in the nervous system. It is imperative that experimental animals are placed in the pen together to receive the same environmental manipulation. Any difference in the sensory experiences of the animals would constitute an unresolvable confound in the interpretation of the subsequently gathered data. It is important to note that animals are only in the run for six hours per day, after which they are returned to pens that provide the minimum square footage per cat required by the USDA. Results from this work are important for the improved treatment of children with visual disorders such as blindness, amblyopia, and strabismus.

A 03-081/A 06-079: Development of Multisensory Integration
Species Used: Male and female domestic shorthair cats *(Felis domesticus)*
Number Used: 2

A 04-127: Development of Multisensory Integration - Cats
Species Used: Male and female domestic shorthair cats *(Felis domesticus)*
Number Used: 4

A 04-154: Experiential Influences on Cross-Modal Development
Species Used: Male and female domestic shorthair cats *(Felis domesticus)*
Number Used: 8

7. The following protocol involves an exception to the ambient temperature requirements for nonhuman primates. The exception was approved by the WFU IACUC, after determination of scientific justification.

A 05-006/A 06-093. Thermoregulation in MDMA Abuse
MDMA or "ecstasy" is a widely known recreational drug of abuse and is commonly abused in warm, crowded environments such as "rave" parties and dance clubs. A major consequence of MDMA use involves elevation of body temperature, which increases the risk of toxicity. Currently there is a critical

lack of understanding of the neuronal processes involved in MDMA abuse and subsequent hyperthermia. This study proposes to study MDMA and the neurohormonal systems in nonhuman primate models. Understanding the mechanism involved in MDMA-induced hyperthermia and the underlying neurochemistry will allow for increased public awareness of the potential toxicities associated with MDMA use and provide new means for treating MDMA-mediated thermogenesis and abuse.

Animals are housed in accordance with USDA regulations and standards. Following instrumentation and acclimation to primate chairs, behavioral experiments are carried out in monkeys in ventilated, sound attenuating chambers designed to accommodate primate chairs. During the experimental sessions, the ambient temperature in the chamber is adjusted as needed to maintain the chamber at temperatures up to 31±1°C. Prior to approving the study, the IACUC met with research and veterinary personnel to develop written procedures designed to ensure the safety of animals. The procedures include specific requirements for monitoring animals during experimental sessions, and outline criteria and a plan for intervention.

Species Used: Male rhesus monkeys (*Macaca mulatta*)
Number Used: 5

Attachment to APHIS FORM 7023

Explanation of Column E Procedures
Wake Forest University 55-R-0001
Fiscal Year 2005-2006

The following protocols involve experiments reported in Column E. Experiments were approved by the Wake Forest University (WFU) Animal Care and Use Committee (ACUC), after their determination of scientific justification.

1. *A05-136: Neurophysiological Recording from Nonhuman Primate Brain*
   Number of animals used in this study: *18*
   Species (common name) of animals used in the study: *Male rhesus monkeys (Macaca mulatta)*
   Explain the procedure producing pain and/or distress:
   One of the aims of this study is to identify those brain areas most affected by sleep deprivation in an animal model. To that end, monkeys are kept awake for 36-54 hours by use of nonaversive stimuli (lights, videos, music, food treats, toys, random computer sounds, interactions with technicians). Behavioral, electrophysiological, and brain metabolic parameters measured after deprivation are compared to baseline parameters. Sleep deprivation studies are performed at minimum 10 day intervals, typically only once per 2-4 weeks per monkey. While the procedures used to produce sleep deprivation are not themselves considered painful, it is recognized that the lack of sleep may cause monkeys to experience some unrelieved distress during the 36-54 hours when they are kept awake. However, such distress is not unrelieved, since the monkeys are allowed to sleep once the physiological and behavioral assessments are completed. In addition, sleep deprivation procedures utilize nonaversive alerting stimuli, constant interaction with technician staff with whom the monkeys are most familiar, and maintainance of social interaction by working with two or more monkeys at a time. Upon completion of the procedure, the monkeys are returned to their home cage and normal social environment and allowed to sleep. They are also given a normal 12 hour sleep cycle that night.

Provide scientific justification why pain and/or distress could not be relieved. State methods used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see next response.):

Although humans can undergo brief periods with little to no sleep, the problems of extended sleep deprivation are vitally important especially for military personnel, shift-workers, medical personnel, mothers with very young children, and long-distance truck drivers, all of whom must continue to do their essential duties with prolonged periods of sleep loss or interruption. Despite such sleep deprivation, societal and job demands require these individuals to be attentive, remember accurately, and make appropriate decisions. Before an effective means of alleviating the effects of sleep deprivation on memory, attention, and decision-making can be developed, it is necessary to identify those cognitive functions and brain areas most affected by sleep deprivation. This ACUC approved study is necessary to characterize the effects of sleep deprivation in an animal model that is quite similar to that of a human performing a job that requires hand-eye coordination, short-term memory, and decisions based on information presented in the task. The experiment provides access to information (i.e. electrophysiological measures and testing of paliative drugs) that cannot be obtained in human studies. The procedures themselves are not painful, but the actual experience of being sleep-deprived may result in distress to the monkeys, which would be unrelieved during the 36-54 hours that they are kept awake. However, since the stress of sleep deprivation is precisely the effect being studied, it cannot be alleviated during the period of the experiment. Efforts are made to alleviate the distress as soon as the experimental measurements are completed, and sleep deprivation is not repeated for a minimum of 10 days.

What, if any, federal regulations require this procedure? Cite the agency, the Code of Federal Regulations (CFR) title number and the specific section number (e.g. APHIS, 9 CFR 113.102):

Agency:

CFR:

This experiment is funded by the Defense Advanced Research Projects Agency (DARPA) as part of "Preventing Sleep Deprivation" contract DAAD19-02-1-0060.